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<b>(21) International Application Number:</b> PCT/US84/00540 <b>(22) International Filing Date:</b> 11 April 1984 (11.04.84) <b>(31) Priority Application Number:</b> 483,508 <b>(32) Priority Date:</b> 11 April 1983 (11.04.83) <b>(33) Priority Country:</b> US  <b>(60) Parent Application or Grant</b> <b>(63) Related by Continuation</b> US 483,508 (CIP) Filed on 11 April 1983 (11.04.83)  <b>(71) Applicant (for all designated States except US):</b> MID-AMERICA DAIRYMEN, INC. [US/US]; P.O. Box 1837, S.S. Station, 800 West Tampa Street, Springfield, MO 65805 (US).		<b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only) :</b> REDDY, Malireddy, S. [US/US]; 1983 Telluride Street, Aurora, CO 80011 (US).  <b>(74) Agents:</b> COLLINS, John, M. et al.; Schmidt, Johnson, Hovey & Williams, 1400 Mercantile Bank Tower, 1101 Walnut Street, Kansas City, MO 64106 (US).  <b>(81) Designated States:</b> AT (European patent), AU, BE (European patent), BR, CH, CH (European patent), DE, DE (European patent), DK, FI, FR (European patent), GB, GB (European patent), JP, LU (European patent), NL, NL (European patent), NO, SE, SE (European patent), US.  <b>Published</b> With international search report.
<b>(54) Title:</b> CHEESE STARTER MEDIA AND METHOD OF MAKING SAME  <b>(57) Abstract</b>  Low cost, readily dispersible, phage-resistant cheese starter media which include milk-derived nutrients (e.g., nonfat milk and whey) along with a minor proportion of preferably free or unbound lecithin. The media also may advantageously include sodium tetraphosphate which assists in the dispersion of whey solids. The media of the invention can be used at significantly lower levels as compared with nonfat dry milk solids (e.g., 7 percent versus 12 percent), while nevertheless obtaining essentially equivalent results in terms of culture growth and final culture properties. A method of producing the media is also disclosed, involving liquid preblending of phosphates and lecithin, followed by addition thereof to milk-derived nutrients and reaction of the phosphates to tie up free calcium ion. The final step involves drying of the mixture to yield a substantially homogeneous, reconstitutable powder. In other cases the phosphate-lecithin preblend can be dried for later addition to milk-derived nutrients to produce a final starter medium.		

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1 CHEESE STARTER MEDIA AND METHOD OF MAKING SAME

5 This is a continuation-in-part of application Serial No. 06/483,508, filed April 11, 1983 and entitled "Cheese Starter Media."

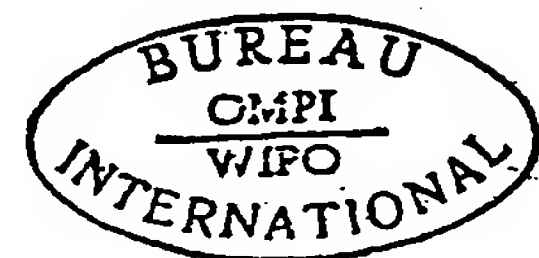
Background of the Invention

1. Field of the Invention

10 The present invention is broadly concerned with novel, low cost cheese starter media which can be used by cheese makers in the growth of bulk starter cultures, along with a unique method of producing the media. More particularly, it is concerned with such media which in preferred forms include a minor amount of free or unbound lecithin, and/or minor quantities of sodium tetra-  
15 phosphate for purposes of imparting a stable, homogeneous dispersion of the starter media ingredients; in its method aspects, the invention involves preparation of a liquid preblend including phosphate anti-bacteriophage agent(s) and, preferably, lecithin, and mixing of the preblend with milk-derived nutrients (e.g., whey and nonfat milk), followed by drying to yield a smooth, consistent, substantially uniform and homogeneous  
20 reconstitutable powder.

2. Description of the Prior Art

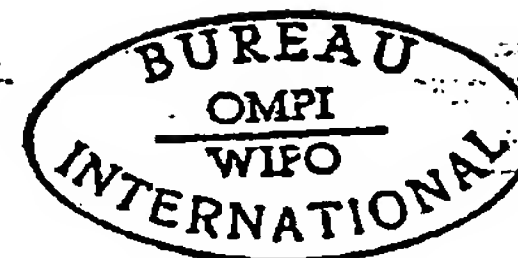
30 In the manufacture of natural cheese, milk in a cheese vat is inoculated with a minor amount (e.g. 2-4 percent) of a bulk starter providing the necessary culture of acid-forming microorganisms used for the particular cheese being manufactured. For example, in the case of Italian cheeses such as mozzarella, it is the  
35 usual practice to employ Streptococcus thermo-



1     philus together with one or more lactobacilli such  
as Lactobacillus bulgaris. In the art, the strep-  
tococci are generally referred to by the short  
name of "coccus", while the lactobacilli are  
5     referred to as "rod" bacteria because of their  
appearance under microscopic examination.

10     The quantity and activity of cheese-  
making microorganisms can be critical to the  
overall outcome of the process and final cheese  
quality. Again referring to Italian cheese, it  
has been found that, in order to make acceptable  
cheese, the ratio of coccus to rod microorganisms  
in the starters should be from about 1:1 to 5:1,  
the most preferable level being about 2:1 to 3:1.  
15     If these ratio considerations are not met, the  
final Italian cheese product may be deficient in  
flavor or physical properties such as elasticity  
and "stringiness."

20     It is the universal practice among  
cheese makers to grow their bulk starters using  
relatively minor amounts of seed culture. In such  
techniques, the seed culture is inoculated into a  
starter medium and allowed to incubate therein so  
that the culture cells will multiply to produce  
25     the desired bulk starter for use in cheese making.  
Here again, the types of starter media and the  
techniques used during the incubation process can  
have a relatively critical outcome on the quality  
of the final bulk starter, and hence on the cheese  
ultimately produced. A dilute dispersion of  
30     nonfat milk (e.g., 12 percent solids level) in  
water has long been considered the starter medium  
of choice. However, use of nonfat milk in this  
context is a relatively expensive proposition, and  
35     therefore cheese makers have in the past sought to



1 use media of a less expensive nature which either  
eliminate nonfat milk entirely, or sharply limit  
its use by provision of substitute materials.

5 For example, U.S. Patent No. 3,852,158  
describes a starter media which includes milk-  
derived materials, a nitrogen source, and citrate  
anion. In preferred forms, the starter media  
described in this patent contain a major amount of  
sweet whey and a minor amount of nonfat dry milk  
solids.

10 U.S. Patent No. 3,998,700 describes  
starter media which include both acid and sweet  
whey solids together with nonfat dry milk solids.  
Finally, U.S. Patent No. 2,805,950 describes the  
15 use of whey for culturing bacterial microorganisms  
used in making a swiss cheese.

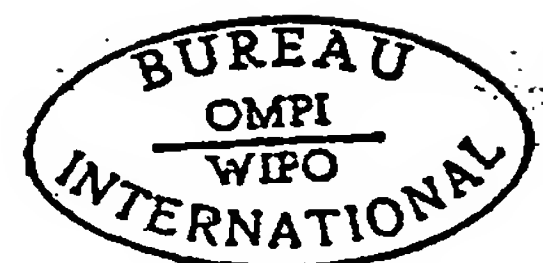
20 While a number of alternative starter  
media have thus been proposed in an attempt to  
provide an acceptable substitute for expensive  
nonfat milk, none of these media have given re-  
sults completely equivalent to that of the nonfat  
milk. In many cases, the alternative media do not  
provide the ideal environment for bacterial  
growth, or in the case of Italian cheese making,  
25 the final coccus to rod ratio obtained may be  
improper. Moreover, in those media which incor-  
porate relatively large quantities of whey, a  
problem arises by virtue of the phenomenon known  
as "whey out." Specifically, large amounts of  
whey in a starter medium can precipitate to the  
30 bottom of the starter tank and create severe  
handling problems. In fact, these problems can  
become so severe that some cheese makers simply  
refuse to use starter media containing substantial  
35 amounts of whey, even if growth characteristics of  
such media are satisfactory.





1 U.S. Patent No. 3,041,248 to Hargrove  
describes the use of various phosphates for the  
control of bacteriophage, which are active against  
lactic acid bacteria. Indeed, the many starter  
5 media presently available include various phos-  
phates for the purpose of combatting bacterio-  
phage. In this connection, it is known that the  
phosphates react with or tie up the readily avail-  
able calcium ion present in the media, and this in  
10 turn prevents the bacteriophage from adsorbing  
onto the specific starter bacterium. In conven-  
tional practice, the phosphates are simply added  
directly to the remaining dry ingredients of a  
starter medium, followed by appropriate blending  
15 and bagging. This conventional dry blending  
procedure presents a number of practical problems  
in the use of starter medium, however, particular-  
ly with respect to the phosphate content thereof.

Specifically, the phosphates tend to be  
20 of irregular, grainy appearance and size in a dry  
condition, and therefore tend to settle out or  
stratify in the dry blended media. When the media  
are reconstituted in water, problems are presented  
not only from the standpoint of solubility (the  
25 conventional media are sometimes difficult to  
disperse in water), but more important the phos-  
phates present may not completely react with free  
calcium ion. In order to ensure the most effec-  
tive use of the phosphate anti-bacteriophage  
30 agents, it is desirable that the dry medium be  
smooth, uniform and substantially homogeneous; and  
this is particularly the case when it is borne in  
mind that the media may be used with radically  
different equipment and cheese-making practices  
35 from manufacturer to manufacturer. Non-uniformity



1 inevitably means that in certain portions of the  
media the phosphate concentration is too low,  
while in other portions it is too high; and both  
of these conditions should be avoided.

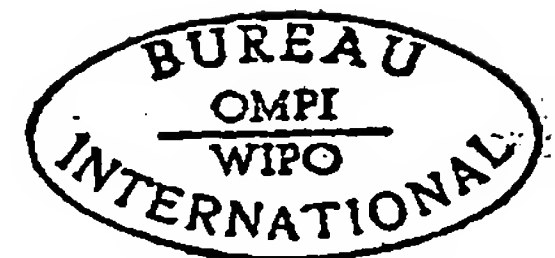
5 In addition, when a typical dry blended  
powder medium is reconstituted in water, it is  
desirable to allow sufficient time for the phos-  
phate to react with available calcium. Under  
normal cheese plant conditions, however, this  
10 reaction time should be minimized, and in some  
instances time constraints have forced cheese  
makers to employ a starter medium which has been  
insufficiently reacted; the result of this is that  
the problem of bacteriophage may not have been  
15 completely eliminated, and this in turn can have  
severe consequences in terms of cheese production.

In short, the irregular, non-uniform  
nature of many dry blended starter media composi-  
tions lead to a number of rather serious problems,  
most particularly with respect to the proper  
20 utilization of phosphate anti-bacteriophage agents  
present therein.

Accordingly, there is a heretofore  
unsatisfied need in the art for less expensive,  
25 alternative starter media, and a method of produc-  
tion thereof, which can be used in lieu of nonfat  
milk per se while giving essentially equivalent  
results in terms of culture growth and qualities,  
and which avoids practical difficulties such as  
30 "whey out" and problems stemming from non-  
uniformity.

#### Summary of the Invention

35 The problems outlined above are in large  
measure solved by the present invention which



1 provides greatly improved starter media for cheese  
making microorganisms. A principal advantage of  
the invention is the fact that dried media can be  
5 readily produced which are virtually homogeneous  
and give the appearance of a fine, talcum-like  
powder. The uniformity of the media of the inven-  
tion facilitates reconstitution and dispersal  
thereof in water or other aqueous media and sub-  
stantially prevents differential phosphate concen-  
10 trations.

In terms of production methods, dried,  
reconstitutable bacteriophage-resistant starter  
media for cheese-making microorganisms are pro-  
duced by first providing a quantity of milk-  
15 derived nutrient such as whey, nonfat dry milk  
solids, or a combination of the foregoing. In the  
next step, a liquid preblend is prepared, sepa-  
rately from the first quantity of milk-derived  
nutrients, with the preblend having a phosphate  
20 anti-bacteriophage agent dispersed therein.  
Advantageously, this preblend also includes a  
quantity of lecithin along with appropriate miner-  
als. The liquid preblend is then added to the  
milk-derived nutrients to form a liquid mixture,  
25 and this mixture is then allowed to react for a  
period of, typically, 1-12 hours in order to  
permit the phosphates to react with available  
calcium ion in the mixture. In the final manu-  
facturing step, the reacted mixture is dried,  
30 usually using conventional spray drying tech-  
niques.

In preferred manufacturing procedures,  
the pH of the milk-derived nutrients is adjusted  
to a level of from about 6.0 to 7.5 prior to the  
35 addition of the liquid preblend. Further, the





1 milk-derived nutrients may be partially concen-  
trated to a solids level of from about 25 to 50  
percent, and the temperature thereof is advan-  
tageously adjusted to from about 35 to 60 degrees  
5 Fahrenheit, in order to facilitate dispersal of  
the preblend therein.

The preblend is normally prepared by  
heating a qauntity of water to a temperature of  
from about 85 to 130 degrees Fahrenheit, followed  
10 by addition of the phosphate agent(s) such as mono  
and disodium phosphate and sodium tetraphosphate  
to the heated water, with agitation to achieve a  
substantially uniform dispersion. In preferred  
procedures, an amount of free or unbound lecithin  
15 is also added to the preblend mixture.

The final dried starter media in accord-  
ance with the invention are very uniform and  
homogeneous, and exhibit extremely desirable  
physical characteristics which greatly facilitate  
20 their use. As noted, such media in dried form  
broadly comprise a milk-derived nutrient and a  
minor amount of unbound lecithin. The milk-  
derived nutrients used in the preferred starter  
media advantageously comprise relatively modest  
25 amounts of nonfat milk, and major proportions of  
whey. Starter media in accordance with the in-  
vention which include substantial whey fractions  
are greatly improved by the addition of a minor  
amount of sodium tetraphosphate therein, which as  
30 noted is added with the other phosphates in the  
liquid preblend. This additive has been found to  
greatly assist in the aqueous dispersion of the  
whey, and largely eliminates the problem of "whey  
out."

35



1           The addition of free or unbound lecithin  
to the starter media hereof has been found to give  
enhanced results in terms of culture growth and  
final bulk starter properties; indeed, the pre-  
5       ferred media of the invention give essentially  
equivalent results, as compared with use of nonfat  
dry milk solids in aqueous dispersion. In fact,  
such equivalent results obtain through the use of  
significantly smaller quantities of the present  
10       media, as compared with NFDM. Specifically, a 7  
percent solids dispersion of the preferred media  
of the invention gives virtually identical re-  
sults, as compared with a 12 percent solids dis-  
persion of NFDM.

15           As used herein, the term "free" or  
"unbound" in conjunction with lecithin refers to  
lecithin in relatively purified form which is  
substantially free of chemical and/or physical  
bonding to other materials. Such free or unbound  
20       lecithin is to be contrasted with, for example,  
lecithin which may be found in products such as  
fresh milk or buttermilk. In such context, the  
relatively minor lecithin content is believed to  
exist as a lipoprotein where the lecithin is in a  
25       complex with the protein. Such association with  
other constituents may have the effect of altering  
the properties of the lecithin in the context of  
starter media; accordingly, use of the free or  
unbound lecithin as herein defined is preferred.

30

35



1           The media of the invention also advantageously include minerals such as manganese chloride and ferrous ammonium sulphate, and a corn steep solids/whey solids stimulant.

5           Although in preferred forms a complete dried starter medium is provided in accordance with the invention, in other instances a dried preblend can be produced for addition to sweet whey or other milk-derived nutrients to produce a  
10          complete starter medium. Such a preblend would comprise a dried, substantially uniform powder having therein respective quantities of a phosphate anti-bacteriophage agent, lecithin and optionally NFDM, corn steep stimulant and minerals  
15          (e.g., ferrous ammonium sulfate and/or manganese chloride). The lecithin is preferably free or unbound as defined, and is advantageously present at a level of from about 0.1 to 0.8 percent by weight (most preferably, 0.5%).

20          Description of the Preferred Embodiments

          In practice, one of the most preferred starter media of the invention is in the form of a dried composition which can be added to an aqueous  
25          system to give a liquid starter medium. This composition includes the following components:



1

TABLE I

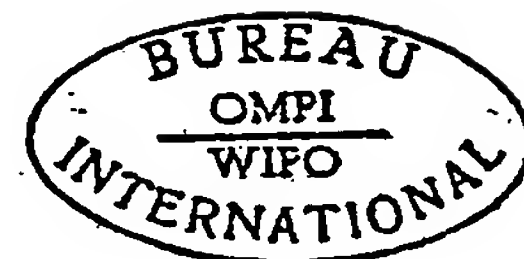
	<u>Ingredient</u>	<u>Initial Quantity</u> <sup>1</sup>	<u>Parts by Wt. of Dried Composition (Dry Basis)</u>
5	Stimulant	700 lbs.	4.120
	Nonfat dry milk solids	900 lbs.	5.290
	Sodium tetra phosphate	800 lbs.	4.700
10	Disodium phosphate	550 lbs.	3.230
	Monosodium phosphate	800 lbs.	4.700
15	Manganese chloride	400 gr.	0.005
	Ferrous ammonium sulfate	400 gr.	0.005
	Lecithin <sup>2</sup>	5 gallons	0.290
20	Sweet liquid whey <sup>3</sup>	280,000 lbs.	77.660

25 <sup>1</sup> Total weight or quantity, including free water, of starting ingredients

<sup>2</sup> Lecithin in liquid form, 50% by wt. solids; or could be in the form of a dried powder

30 <sup>3</sup> May alternatively be derived by mixing 13,198 lbs. of dried whey with 266,802 lbs. of water

35



1           The preferred dried powder media composition is made as follows. In the first step, 700  
pounds of the stimulant (a dried mixture of corn  
steep solids and sweet whey solids described in  
5 detail below), along with 900 pounds of the nonfat  
dry milk solids are mixed with the 280,000 pounds  
of liquid sweet whey (either raw or pasteurized,  
normally pasteurized). After sufficient mixing to  
disperse the stimulant and milk solids, the mixture  
10 is neutralized by the addition of sodium  
hydroxide (50%) to a pH of 6.0-7.5 (preferably  
7.0). The pH adjusted mixture is then thermally  
evaporated under vacuum conditions to a 25-50  
percent solids level (preferably 41%), whereupon  
15 the mixture is cooled to 35-60 degree Fahrenheit  
(preferably 50° F.) and transferred to a final  
mixing tank.

A phosphate/minerals/lecithin premix is  
prepared separately from the mixture of stimulant,  
dried milk and whey. This premix is made by  
20 adding 375 gallons of water at 85-130 degrees  
Fahrenheit (preferably 96° F.) to a 1,000 gallon  
mixing tank. Next, 800 pounds of sodium tetra  
phosphate is added, followed by 800 pounds of  
25 monosodium phosphate and 550 pounds of disodium  
phosphate, all with constant agitation. The next  
step involves dissolving the 400 grams of ferrous  
ammonium sulfate and 400 grams of manganese chloride  
in a small amount of water, whereupon these  
30 minerals are added to the agitated mixture of  
water and phosphates. The 5 gallons of lecithin  
is then added to the premix tank, again with  
sufficient agitation to ensure homogeneity. Other  
phosphate anti-bacteriophage agents can of course  
35 be employed in place of the preferred phosphates



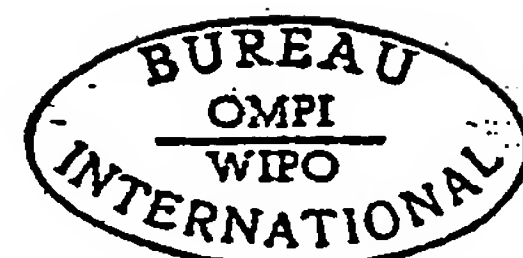


1 listed above, e.g., ammonium phosphates. More-  
over, while dry granular phosphates can be added  
and dissolved, in other instances these can be  
made in plant by mixing the starting ingredients,  
5 e.g., appropriate quantities of phosphoric acid  
and ammonia can be reacted to form the desired  
ammonium phosphates, and the products used direct-  
ly without an intermediate drying step.

10 This premix is then added to the mixture  
of whey, stimulant and dried milk solids, where-  
upon the overall mixture is agitated for 1-12  
hours, and preferably overnight, in order to  
assure that the phosphates react with available  
calcium ion in the mixture. The reacted mixture  
15 is then spray dried to about 3-6 percent moisture  
(preferably 4%) to yield a substantially uniform  
and homogeneous, flowable, dried, powder-like  
material. Of course, if the medium is made in the  
plant where it is to be used, it may be advanta-  
geous not to dry the medium but rather to use the  
20 same directly with appropriate dilution to achieve  
the desired solids content for the final liquid  
medium, i.e., from about 5 to 15 percent, and more  
preferably from about 7-11 percent.

25 In other cases, however, the phosphate/  
minerals/lecithin premix can be formulated alone  
and dried, whereupon it can later be added to whey  
or other milk-derived nutrients to yield a final  
medium. This procedure gives some of the advan-  
30 tages of the preferred techniques described above,  
and avoids the expense of drying, storing and  
shipping of large quantities of whey and the other  
components of the complete medium.

35 The stimulant referred to above is made  
by taking 280,000 pounds of separated raw whey

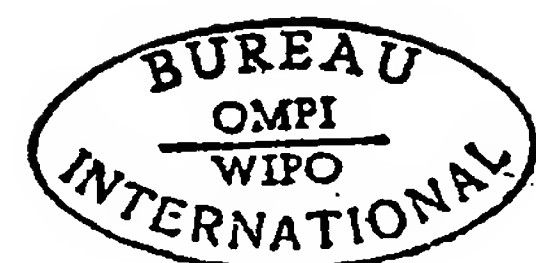


1 from the cheese-making vat (such amount of whey  
being a separate quantity from that used in the  
starter media per se listed in Table I), and  
5 adjusting the pH thereof to a level of about 8.0  
with sodium hydroxide. The pH-adjusted whey is  
then evaporated to a 34 percent solids level, and  
cooled to 50 degrees Fahrenheit. The evaporated  
whey is then pumped into a tank containing 42,880  
10 pounds of commercially purchased corn steep liquor  
having a pH of 4.15. Such liquor is obtained from  
The Staley Corporation of Decatur, Illinois, and  
has a 50 percent solids level. This creates a  
mixture containing about 60 percent by weight corn  
steep solids and 40 percent by weight whey solids.  
15 The 60 percent-40 percent mixture is then agitated  
overnight, filtered and spray dried to about 4  
percent moisture. The resultant dried product is  
stored in 50 pound bags for subsequent use in the  
starter media. An alternate stimulant can be  
20 produced by replacing the corn steep liquor with  
liquid yeast extract or any other suitable liquid  
stimulant.

25

30

35



1           The above described dried media composi-  
tion can be used to obtain starter media for  
various types of microorganisms used in cheese  
making. For example, in order to obtain a starter  
5   media specifically designed for culturing micro-  
organisms used in making Italian cheeses such as  
mozzarella (referred to as "coccus" and "rod"  
microorganisms), the following procedure is fol-  
lowed. First, 3,950 pounds (476 gallons) of fresh  
10   water is pumped into the starter tank, and the  
water is heated to a level of 100-110 degrees  
Fahrenheit. Three hundred pounds of the dried  
starter medium of Table I above is next added to  
the water, with the aid of a powder horn. The pH  
15   of this mixture will be about  $6.6 \pm 0.1$ . The  
mixture is then heated to 190 degrees Fahrenheit  
and held for one hour at this temperature, where-  
upon the mixture is cooled to a temperature of  
100-112 degrees Fahrenheit. At this point, the  
20   mixture is ready for inoculation with the desired  
coccus and rod cultures.

          In another example, a dried composition  
very similar to that described above can be em-  
ployed to prepare a culture medium for lactic  
25   cultures to be used in making American-type chees-  
es. From a compositional standpoint, the only  
difference involves the addition of a total of  
2200 grams of ferrous sulfate. In this technique,  
3,950 pounds (476 gallons) of fresh water is  
30   pumped into the starter tank, and is heated to a  
temperature of 100-130 degrees Fahrenheit. Three  
hundred pounds of the modified dried starter  
medium is next added to the heated water with the  
aid of a powder horn, giving a resultant pH of the  
35   mixture of approximately  $6.6 \pm 0.1$ . The mixture



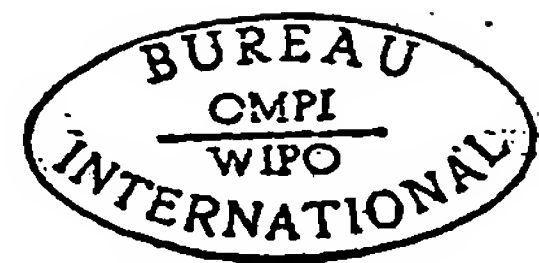
1 is then heated to 190 degrees Fahrenheit, and held  
at this temperature for 1 hour, whereupon the  
medium is cooled to 74-80 degrees Fahrenheit.  
Here again, at this point in the procedure, the  
5 medium is ready for inoculation with the appropriate lactic cultures.

While in many instances the preparation  
of a complete, dried composition of the type  
described above is advantageous for reasons for  
ease of handling or the like, the invention is not  
10 so limited. That is to say, in appropriate circumstances a cheese manufacturer may wish to use whey derived directly from the cheese making operation, as opposed to having whey in the dried media composition. In such a case, one option  
15 would be to prepare a supplement mixture which can be added to liquid whey to produce a final liquid starter media. The stimulant, phosphates, minerals, nonfat dry milk and lecithin are premixed either by dry or wet blending, and are added to  
20 liquid whey. The pH of the mixture is then adjusted to a level of 6.6-6.7 through addition of, e.g., sodium hydroxide or ammonia, and the mixture is heated to 190 degrees Fahrenheit and held at  
25 that temperature for one hour. The mixture is then cooled to 100-112 degrees Fahrenheit, whereupon it is ready for inoculation with a desired culture.

30

EXAMPLE 1

In this test a comparison was made  
between the two starter media, namely nonfat dry  
milk solids, and the most preferred lecithin-  
35 containing composition of the invention, in terms



1 of final coccus/rod ratios, bacterial counts and  
the activities achieved using the respective  
media.

5 The nonfat dry milk was reconstituted in  
water at 12.0% solids in water, whereas the medium  
hereof (Table I) was reconstituted in water at a  
level of only 7 percent solids. Both media were  
heat treated by heating to 190 degrees Fahrenheit  
and holding at this temperature for one hour. The  
10 media were then cooled to 102 degrees Fahrenheit  
and inoculated with identical quantities (1%) of  
coccus and rod cultures (Streptococcus thermo-  
philus and Lactobacillus bulgaris). The cultures  
were then incubated in the respective media at 102  
15 degrees Fahrenheit until the titratable acidity  
exceeded 1.0; for the NFDM system this took about  
5.5 hours, and for the medium of the invention  
about 7 hours. At this point the cultures were  
cooled to 40 degrees Fahrenheit. The two cultures  
20 were then tested for titratable acidity, coccus/  
rod ratio, pH and activity, all using conventional  
techniques. The results of this test were:

TABLE II

Media	Final pH	Final Titratable Activity	Coccus/Rod Ratio	Total Bacterial Count	Activity
NFDM (12%)	4.20	1.02	4:1	$140 \times 10^7$	0.70
Inven- tion (7%)	4.35	1.02	4:1	$130 \times 10^7$	0.72





1           As noted in Table II, the results using  
the costly NFDM are very similar to those obtained  
with the medium of the invention. This is very  
5       surprising in that a significantly greater quantity  
of NFDM was employed (12%) versus the invention  
(7%). At present day typical retail costs, the  
cost of using the medium of the invention to  
achieve equivalent results is on the order of only  
10       50 percent of that of using NFDM as a starter  
medium.

15           In another similar test, equal percentage  
solids amounts of NFDM and the medium of the  
invention were tested (7% solids used in both  
cases). These tests were conducted in the same  
manner as heretofore described except that the 7  
percent solids NFDM media was incubated after  
inoculation until the pH reached about 4.25; this  
is the pH level where NFDM systems normally give a  
titratable acidity of greater than 1.0. That is  
20       to say, if a 7% solids NFDM system is allowed to  
incubate to a 1.0% or greater titratable acidity,  
the pH would be abnormally low (e.g., around 3.7),  
and the bacteria would be injured or the coccus/  
rod ratio would be totally unacceptable.

25           Accordingly, the incubation of the 7  
percent NFDM system was terminated at the normal  
pH achieved for full strength NFDM media, which  
took about 5 hours. On the other hand, the medium  
of the invention was incubated for a period of  
30       about 7 hours until the titratable acidity ex-  
ceeded 1.0. The final results were:

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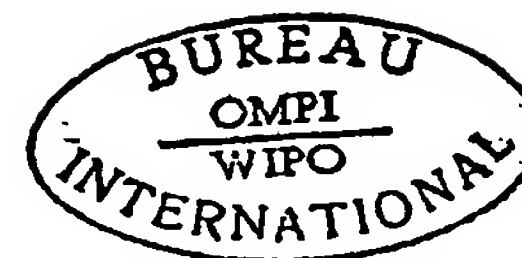


TABLE III

Media	Final pH	Final Titratable Activity	Coccus/Rod Ratio	Total Bacterial Count	Activity
NFDM (7%)	4.25	0.79	3:1	$68 \times 10^7$	0.58
Inven- tion (7%)	4.45	1.01	4:1	$120 \times 10^7$	0.71

Thus, the low solids NFDM system proved deficient in titratable acidity, bacterial count, and activity, as compared with the invention. Moreover, on a cost basis the medium of the invention is far superior, even at equal solids levels.

EXAMPLE 2

In this example, a comparative test was made between a medium in accordance with the invention (Table I) prepared using the preblending and drying procedure of the invention, versus a compositionally identical medium made simply by dry blending all of the ingredients (the lecithin used in this case was also a dried powder). The separate media were then reconstituted in 60 degrees Fahrenheit water, with agitation as necessary to a 7 percent solids level, and cooked at 190 degrees Fahrenheit for one hour. The media were then cooled to 104 degrees Fahrenheit and inoculated with 0.1 ml. of milk grown coccus and rod culture.

The inoculated media were then incubated until the pH thereof dropped to 4.8 (about 5 1/2 hours), whereupon the pH was raised to 6.2 by the



1 addition of 50 percent sodium hydroxide. The  
incubations were then allowed to continue until  
the titratable acidity in both cases was 1.10.  
5 With the liquid preblend, spray dried medium of  
the invention, this took a total of about 10 1/2  
hours, whereas with the dry blended medium a total  
incubation time of 11 1/4 hours was required.

At this point the media were cooled to  
55 degrees Fahrenheit and tested as set forth in  
10 certain entries of the following Table:

15

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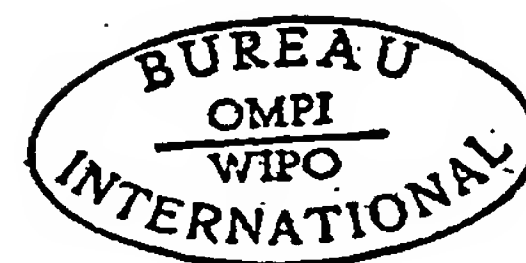
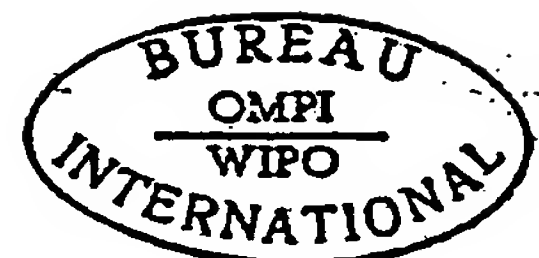


TABLE IV

COMPARISONS OF LIQUID PREBLENDING AND DRYING vs.  
SIMPLE DRY BLENDING ON THE PHYSICAL AND CULTURE  
GROWTH-SUPPORTING PROPERTIES OF STARTER MEDIA

Comparison Number	Characteristics	Liquid Pre- blend Spray Dried Medium	Dry Blended Medium
1.	Physical Appearance	Smooth and uniform-- similar to talcum powder	Gritty and Irregular sizes of the various in- gredients evident
2.	Stratification of the media	None	Stratified-- Phosphates Tend to settle down in the powder
3.	Solubility	Instantly soluble even in the 60° F. water	5 minutes agitation re- quired to solubilize in the 60° F. water
4.	Initial pH of the powdered medium when reconstituted	6.65	6.30
5.	Precipitation upon reconstitu- tion and heating	None	Consider- visible pre- cipitates settled to bottom
6.	Time to grow coccus and rod culture using the 1 step neu- tralization to arrive at final 1.10 titratable acidity	10 1/2 hrs.	11 1/4 hrs.



Comparison Number	Characteristics	Liquid Pre- blend Spray Dried Medium	Dry Blended Medium
7.	Coccus/rod ratio after growth	3:1	1:1
8.	Total bacterial count per gram after culturing	$230 \times 10^7$	$200 \times 10^7$
9.	Activity measured in terms of titrat- able acidity	0.65	0.63
10.	Smoothness of liquid medium after growth of culture	Smooth	Grainy--an appearance of buttermilk

The foregoing Table demonstrates the many advantages obtained through use of the liquid preblend-drying procedure for producing starter media. The smooth, uniform, essentially homogeneous nature of the media of the invention not only facilitates quick, easy handling, but also gives measurably enhanced results in terms of desirable coccus/rod ratios, bacterial counts and activities.

### EXAMPLE 3

This example sets forth another preferred media composition in accordance with the invention, which has been formulated to reduce the incubation time needed to reach a final titratable acidity level of about 1.10 from an average of 10 1/2 hours to about 7 1/2-8 1/2 hours.

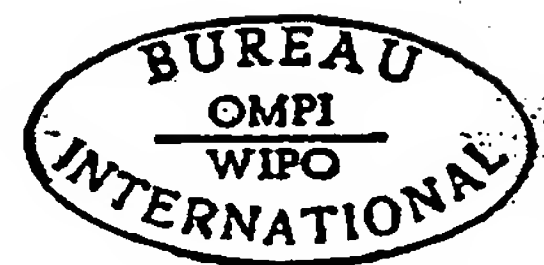




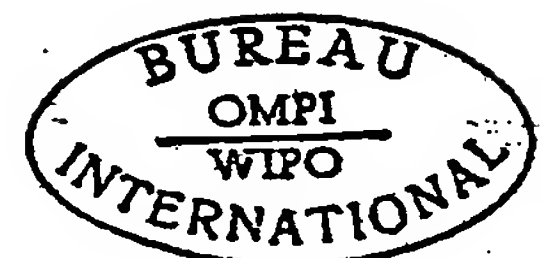
TABLE V

<u>Ingredient</u>	<u>Quantity Used</u>
<sup>1</sup> Yeast extract	850 lbs.
5 Nonfat dry milk powder	1,000 lbs.
Disodium phosphate	1,479 lbs.
Monosodium phosphate	221 lbs.
Ferrous ammonium sulfate	17 lbs.
<sup>2</sup> Manganese chloride	450 grams
Lecithin	2 1/2 gallons
Sweet liquid whey	Balance to yield 17,000 lbs. batch of dried medium

<sup>1</sup> Employed as a stimulant

<sup>2</sup> 50% solids liquid lecithin

The medium of Table IV is made as outlined above in Example 1. In the first step the liquid whey, NFDM powder and yeast extract are mixed and ammonium hydroxide or anhydrous ammonia is added to adjust the pH to 7.0, followed by drying to about 40 percent solids and cooling to 50 degrees Fahrenheit. A liquid preblend of lecithin, the phosphates and the minerals is then made by dispersing these ingredients in 96 degrees Fahrenheit water as outlined in Example 1. The preblend is then added to the cooled nutrients, and the mixture is allowed to react overnight, and the pH is then adjusted as necessary using  $\text{NH}_4\text{OH}$  or anhydrous ammonia. Finally, the mixture is spray dried to about 4% moisture.



1

EXAMPLE 4

5

A highly preferred lactic culture medium for growing cheddar cheese starter microorganisms is set forth below:

TABLE VI

	<u>Ingredient</u>	<u>Quantity Used</u>
10	<sup>1</sup> Corn steep solids	1,360 lbs.
	<sup>1</sup> Yeast extract	425 lbs.
	Nonfat dry milk powder	1,000 lbs.
	Sodium tetraphosphate	840 lbs.
	Disodium phosphate	435 lbs.
	Monosodium phosphate	1,275 lbs.
	Manganese chloride	250 grams
15	<sup>2</sup> Ferrous ammonium sulfate	10 lbs.
	<sup>2</sup> Lecithin	2 1/2 gallons
	Sweet liquid whey	Balance to yield a 17,000 lb. batch of dried medium
<hr/>		
20	<sup>1</sup> Stimulants	
	<sup>2</sup> 50% solids liquid lecithin	

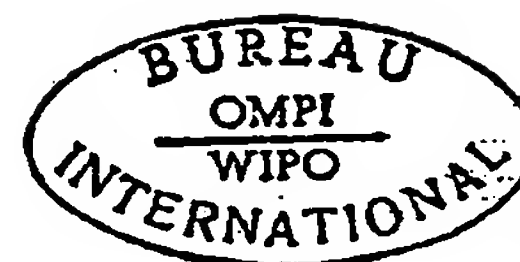
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This medium is made as outlined above, wherein the corn steep solids, yeast extract, NFDM and whey are initially mixed, and a separate liquid preblend of the phosphates, minerals and lecithin in water is added thereto, followed by overnight reaction and drying.

30

The tests described in the foregoing examples were performed as follows:

35



- 1     pH     Hydrogen ion concentration was determined  
             using Beckman pH meter

Titratable Acidity

- 5           9 grams of the medium sample was titrated  
             with 0.1 N sodium hydroxide using pheno-  
             phthalin as an indicator. A faint pink color  
             indicated the end point.

Coccus and Rod Ratio

- 10           A one in ten dilution of culture in water was  
             smeared on a clean glass slide, stained with  
             methylene blue, and examined under a compound  
             microscope. The ratio was determined on the  
             basis of clump and individual counts.

Total Bacterial Count

- 15           The cultured samples were serially diluted in  
             sterile phosphate buffered water according to  
             the procedures outlined in the Standard  
             Methods for the examination of dairy products  
             and plated using tryptic soy agar fortified  
20           with 0.5 percent yeast extract. The plated  
             samples were incubated at 37 degrees Centi-  
             grade for 4 days. The counting and expres-  
             sion of the test results were done according  
             to the Standard Procedures.

25           Activity Test

- 30           2 grams of culture was inoculated into 100  
             ml. of sterile 10.0 reconstituted nonfat dry  
             milk. The nonfat dry milk was pretested for  
             the inhibitory compounds. The inoculated  
             milk was incubated at 36 degrees Centigrade  
             for 45 minutes. At the end of incubation,  
             the temperature was gradually increased to 46  
             degrees Centigrade within a span of 30 min-  
             utes and it was thereafter maintained at that  
35           temperature for a period of 1 hour. The



1 samples were then chilled to prevent any  
further acid development. Ten grams of the  
sample was carefully weighed into a 25 ml.  
5 beaker. Ten drops of indicator (pheno-  
phthalein) was added and the entire contents  
were titrated against 0.1 N sodium hydroxide  
until a faint pink color persisted for 15  
seconds. The results were expressed as  
percent titratable acidity.

10 Although the above described specific  
culture media are preferred in terms of composi-  
tion and method of preparation thereof, those  
skilled in the art will readily appreciate that  
15 the invention is not so limited. That is to say,  
in other forms of the invention, various substi-  
tute and/or additional materials may be employed,  
as opposed to those specifically recited in Tables  
I, V and VI, and moreover the amounts of use can  
20 be varied for the respective components. To give  
but a few examples, the preferred corn steep  
solids/sweet whey solids stimulant can be employed  
in an amount from about 1-10 percent by weight,  
and more preferably from about 2-6 percent by  
25 weight (dry basis). Other possible stimulants  
useful in this context include yeast extract,  
hydrolyzed vegetable proteins, pancreatic enzyme  
digests, and protease-treated milk. Such stimu-  
lants serve as non-specific growth rate enhancers  
30 which increase the rate of acid production and  
growth. Stimulants of this type have been known  
in the past, particularly in the context of start-  
er media which contain other materials besides  
strictly nonfat dry milk solids.

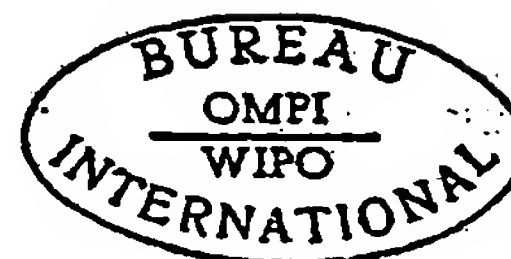
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1           While the use of nonfat dry milk solids  
and whey is preferred in order to provide the  
requisite milk-derived nutrients for the overall  
media, other materials such as casein, the various  
5   caseinates, casein hydrolyzates, partially de-  
mineralized whey, and whey protein concentrates  
could also be used. In those instances where  
nonfat milk is employed, such should be present at  
a level of from about 1 to 10 percent by weight,  
10   and more preferably from about 4 to 6 percent by  
weight (dry basis). In like manner, when whey is  
employed, such should be present at a level of  
from about 50 to 90 percent by weight, and more  
preferably from about 70 to 80 percent by weight  
15   (dry basis).

As noted above, the use of sodium tetra-  
phosphate in the media of the invention is pre-  
ferred, particularly in those instances where a  
major proportion of whey is present. This serves  
20   to minimize the extent of so-called "whey-out" by  
aiding in the dispersion of the whey solids.  
Advantageously, the sodium tetrphosphate is  
present at a level of at least about 2 percent by  
weight, and more preferably from about 3 to 13  
25   percent by weight (dry basis).

The disodium phosphate and monosodium  
phosphate additives are employed in the preferred  
composition in order to inhibit bacteriophage.  
The monosodium phosphate should be used at a level  
30   of from about 2 to 8 percent by weight (dry ba-  
sis), whereas the disodium phosphate should be  
used at a level of about 3 to 13 percent by weight  
(dry basis). Moreover, the combination of sodium  
tetrphosphate with disodium phosphate and mono-  
35   sodium phosphate is particularly preferred inas-





1 much as this combination gives good whey dispersi-  
bility, bacteriophage protection, and a buffering  
capacity in the overall system.

5 While manganese chloride and ferrous  
ammonium sulfate have been employed in minor  
amounts as additive minerals, it will be readily  
seen that other minerals and levels of use can be  
employed. Particular minerals and optimum levels  
of use thereof are within the skill of the art.

10 The free or unbound lecithin forming a  
part of the preferred media of the invention  
should be present at a level of from about 0.05 to  
25 percent by weight, and more preferably from  
about 0.20 to 1 percent by weight (dry basis).  
15 The utility of free or unbound lecithin in the  
media of the invention is not fully understood,  
but it is believed possible that the presence of  
lecithin (a phospholipid) improves the cellular  
integrity of the cheese-making microorganisms and  
thus promotes growth. In addition, lecithin is  
20 known to be an emulsifier, and could assist in the  
transport of nutrients into the cellular structure  
of the microorganisms. However, it will be appre-  
ciated that the foregoing represents hypothesis,  
and there is no wish to be bound to any sort of  
25 theory of operability in connection with use of  
lecithin.

During use of the media in accordance  
with the invention, it is desirable that the final  
30 liquid medium have a solids content of from about  
5 to 15 percent by weight and more preferably from  
about 7-12 percent by weight, and most preferably  
from about 7-8 percent by weight. Obviously, use  
of the smallest amount of solids is preferred for  
35 economic reasons.



1           A variety of culture-growing techniques  
can be employed with use of the media of the  
invention. As noted above, the traditional tech-  
5       nique of inoculating the medium with microorgan-  
isms at a starting pH in the range of, typically,  
6.0-6.5, followed by incubation until the medium  
exhibits a pH in the range of 4.0-4.5, gives  
excellent results. In addition, certain other pH  
10       modification techniques described in recent years  
(see, e.g., U.S. Patent No. 4,282,255) can be used  
to good effect in conjunction with the improved  
media of the invention.

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1     Claims

1.     A method of making a dried, re-constitutable, bacteriophage-resistant starter medium for cheese-making microorganisms, said method comprising the steps of:

5     providing a first quantity of milk-derived nutrient;

preparing, separately from said first quantity of milk-derived nutrient, a liquid preblend having a phosphate anti-bacteriophage agent dispersed therein;

10     adding said liquid preblend to said milk-derived nutrient to form a liquid mixture, and allowing said agent to react with available calcium ion in said mixture; and

15     drying the reacted mixture.

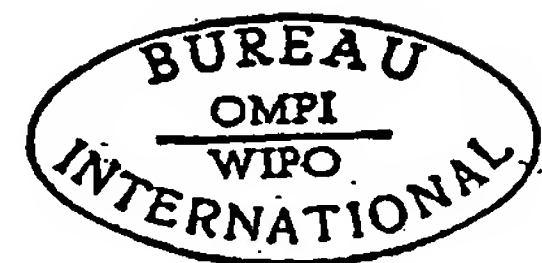
2.     The method of Claim 1, said nutrient comprising sweet whey.

3.     The method of Claim 1, said nutrient comprising nonfat milk.

25     4.     The method of Claim 1, including the step of adjusting the pH of said first quantity of milk-derived nutrient to a level of from about 6.0 to 7.5.

30     5.     The method of Claim 1, including the step of drying said first quantity of milk-derived nutrient to a level of from about 25 to 50 percent solids.

35



1           6. The method of Claim 5, including  
the step of adjusting the temperature of the dried  
nutrient to a level of from about 35 to 60° F.

5           7. The method of Claim 1, said pre-  
blend preparation step comprising the steps of:  
heating a quantity of water to a temperature  
of from about 85 to 130° F.; and  
adding said agent to the heated water, and  
10           agitating the resultant mixture to  
substantially uniformly disperse the  
agent therein.

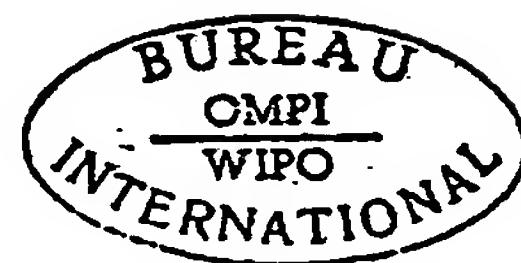
15           8. The method of Claim 7, said agent  
being selected from the group consisting of mono-  
sodium phosphate, disodium phosphate, sodium  
tetraphosphate and mixtures thereof.

20           9. The method of Claim 1, including  
the step of agitating the admixed preblend and  
milk-derived nutrient prior to said drying step.

25           10. The method of Claim 1, including  
the step of allowing said agent to react for a  
period of from about 1 to 12 hours prior to said  
drying step.

30           11. The method of Claim 1, said leci-  
thin being present at a level of from about 0.2 to  
1.0% by weight in the final dried medium.

35           12. The method of Claim 1, there being  
from about 50 to 90% by weight whey solids and  
from about 1 to 10% by weight nonfat milk solids  
in the final dried medium.



1           13. A dried, reconstitutable, bacterio-  
phage-resistant starter medium made by the method  
of Claim 1.

5           14. A preblend for addition to a milk-  
derived nutrient to produce a starter medium for  
cheese-making microorganisms, said preblend com-  
prising a dried, substantially uniform powder  
10          having therein respective quantities of a phos-  
phate anti-bacteriophage agent, lecithin, one or  
more stimulants and one or more minerals.

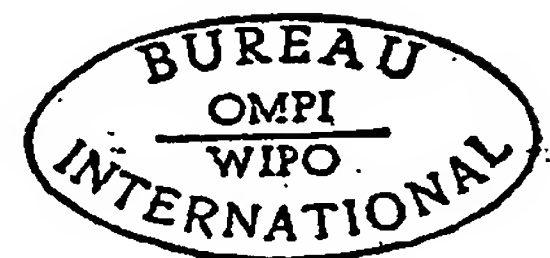
15          15. The preblend of Claim 14, said  
agent being selected from the group consisting of  
monosodium phosphate, disodium phosphate, sodium  
tetraphosphate and mixtures thereof.

20          16. The preblend of Claim 14, said  
lecithin being unbound lecithin.

25          17. The preblend of Claim 14, said  
lecithin being present at a level of from about  
0.1 to 0.8% by weight.

30          18. A starter medium for cheese-making  
microorganisms comprising a dried powder including  
whey and a minor amount of sodium tetraphosphate  
therein for assisting in the aqueous dispersion of  
said whey.

35          19. The medium of Claim 18, said sodium  
tetraphosphate being present at a level of from  
about 3 to 13% by weight on a dry basis.



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20. The medium of Claim 18, including  
respective minor amounts of monosodium phosphate  
and disodium phosphate.

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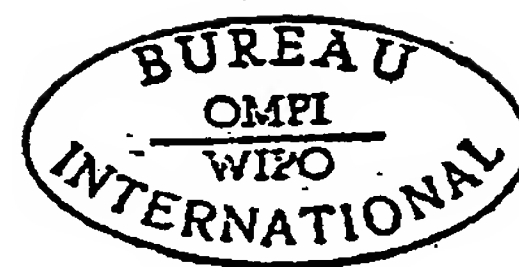
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# INTERNATIONAL SEARCH REPORT

PCT/US84/00540  
International Application No.

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>5</sup> According to International Patent Classification (IPC) or to both National Classification and IPC Int. Cl. <sup>8</sup> C12N 1/20; A23C 21/00; 19/00; 21/02; 9/12 U.S. Cl. 435/253; 426/583; 36, 41, 43						
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched <sup>4</sup></div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 20%; border-bottom: 1px solid black;">Classification System</th> <th style="border-bottom: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">U.S.</td> <td style="padding: 5px;">435/253; 426/580, 582, 583, 34, 36, 42, 43</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; padding-top: 5px; margin-top: 5px;">           Documentation Searched other than Minimum Documentation            to the Extent that such Documents are Included in the Fields Searched <sup>6</sup> </div>			Classification System	Classification Symbols	U.S.	435/253; 426/580, 582, 583, 34, 36, 42, 43
Classification System	Classification Symbols					
U.S.	435/253; 426/580, 582, 583, 34, 36, 42, 43					
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <sup>14</sup>						
Category <sup>7</sup>	Citation of Document, <sup>15</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No. <sup>18</sup>				
A, P	US, A, 4,402,986, published 06 September 1983 Sinkoff et al.	1-20				
A	US, A, 3,354,049, published 21 November 1967 Christensen, V.W.	1-20				
A	US, A, 3,192,124, published 29 June 1965, Kheshgi, S., see Col. 6, lines 23-24.	1-20				
A	US, A, 4,115,199, published 19 September 1978, Porubcan et al. see Col. 3, lines 40-50.	1-20				
A	US, A, 4,289,788, published 15 September 1981, Cajigas, S.D., See Col. 6, lines 53-60	14,16,17				
A	US, A, 4,289,789, published 15 September 1981, Cajigas, S.D., See Col. 6, lines 4-11	14,16,17				
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>15</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p> </div> </div>						
<b>IV. CERTIFICATION</b>						
Date of the Actual Completion of the International Search <sup>2</sup>  <div style="text-align: center; font-size: 1.2em;">28 June 1984</div>	Date of Mailing of this International Search Report <sup>3</sup>  <div style="text-align: center; font-size: 1.2em;">02 JUL 1984</div>					
International Searching Authority <sup>1</sup>  <div style="text-align: center; font-size: 1.2em;">ISA/US</div>	Signature of Authorized Officer <div style="text-align: center;">David M. Naff </div>					

**FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET**

A, P	US, A, 4,382,965, published 10 May 1983, Sandine et al.	1-20
A	US, A, 4,282,255, published 04 August 1981, Sandine et al.	1-20
A	US, A, 3,041,248, published 26 June 1962, Hargrove et al.	1-20
A	US, A, 4,020,185, published 26 April 1977, Andersen et al.	1-20

**V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>10</sup>**

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers \_\_\_\_\_, because they relate to subject matter <sup>12</sup> not required to be searched by this Authority, namely:

2. ☐ Claim numbers \_\_\_\_\_, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out <sup>13</sup>, specifically:

**VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>11</sup>**

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, <sup>16</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No <sup>18</sup>
A	US, A, 4,053,642, published 11 October 1977, Hup et al.	1-20
A	US, A, 3,998,700, published 21 December 1976, Reinbold et al.	1-20
A	US, A, 4,372,979, published 08 February 1983, Reinbold et al.	1-20
A	N, Journal of Dairy Science, Vol. 44, issued July-December 1961, Hargrove et al., Phosphate Heat Treatment of Milk To Prevent Bacteriophage Proliferation in Lactic Cultures, pages 1790-1810, see page 1800, 6th paragraph	1-20
A	N, Utah Science, issued Winter 1979, Richardson et al., USU Lactic Culture System, pages 94-99:	1-20